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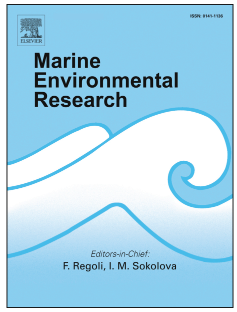

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Changes in the biochemical and nutrient composition of seafood due to ocean acidification and warming

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Abstract:

Ocean acidification and warming may threaten future seafood production, safety and quality by negatively impacting the fitness of marine species. Identifying changes in nutritional quality, as well as species most at risk, is crucial if societies are to secure food production. Here, changes in the biochemical composition and nutritional properties of the commercially valuable oysters, *Magallana gigas* and *Ostrea edulis*, were evaluated following a 12-week exposure to six ocean acidification and warming scenarios that were designed to reflect the temperature (+3°C above ambient) and atmospheric pCO_2 conditions (increase of 350 – 600ppm) predicted for the mid- to end-of-century. Results suggest that *O. edulis*, and especially *M. gigas*, are likely to become less nutritious (i.e. containing lower levels of protein, lipid, and carbohydrate), and have reduced caloric content under ocean acidification and warming. Important changes to essential mineral composition under ocean acidification and warming were evident in both species; enhanced accumulation of copper in *M. gigas* may

be of concern regarding consumption safety. In light of these findings, the aquaculture industry may wish to consider a shift in focus toward species that are most robust to climate change and less prone to deterioration in quality, in order to secure future food provision and socio-economic benefits of aquaculture.

Keywords: Oyster; living resources; biochemistry; food security; global change; environmental stress; multi-stressors; *Magallana gigas; Crassostrea gigas*

1 Introduction

Seafood is the source of >15% of animal protein consumed globally, yet climate change is of increasing threat to the security of this resource (FAO, 2014: Golam et al., 2017; Rice and Garcia, 2011). This, as well as the continued burgeoning human population (Gerland *et al.*, 2014; United Nations, 2015), especially in coastal areas (Firth *et al.*, 2016), are placing increasing and arguably unsustainable demands on sources of animal protein, which are unlikely to be met by land farming (Campbell *et al.*, 2017; Cooley *et al.*, 2012; Delgado, 2003). Some argue the marine environment can make up the shortfall via a 'Blue Revolution'. But as overfishing, habitat destruction, and climate change are already causing decline in fish stocks in many areas (Macura *et al.*, 2016; McCauley *et al.*, 2015; Pauly *et al.*, 1998), there is growing concern about the resilience of the marine environment to withstand increased anthropogenic pressure and provide sustainable food provision in the future (Knights *et al.*, 2015; Porter *et al.*, 2014; UNEP, 2010; Weatherdon *et al.*, 2016).

Aquaculture is increasingly promoted as an alternative to land-based meat production and a solution for securing food provision in the future (Gentry *et al.*, 2017; Naylor *et al.*, 2000; Tacon & Metian, 2013). The aquaculture industry is the fastest growing food sector, with production increasing nearly year-on-year since the 1950s (FAO, 2016), which has now surpassed that of capture fisheries. Molluscan aquaculture is increasingly important, with many molluscs strategically chosen due to their low production cost compared to that of other fish and shellfish due to no requirement for feed (Tacon & Metian, 2013; Troell *et al*. 2014). In 2015, ~15% of the total aquaculture production volume was attributed to molluscan aquaculture (over 16 million tonnes; worth over US$18billion) (FAO 2018). Additionally, mollusc aquaculture has been found as having the lowest environmental production impacts of all animal source food, and therefor may constitute a more sustainable source of protein (Froehlich *et al.*, 2018; Hilborn *et al.*, 2018).

The increased prevalence of obesity in several regions of the world (Abarca-Gómez *et al.*, 2017) is leading to greater public awareness and desire to consume a healthy and balanced diet. A healthy diet should include sufficient proteins, amino acids, essentials fats such as long-chain omega-3 fatty acids, vitamins and minerals (FAO, 2016; Simopoulos, 2002). The proximate composition can be used as a measure of nutritional quality (see EFSA NDA Panel, 2014; Hart & Fisher, 1971; Nielsen, 2006; Tate *et al.*, 2017), dividing the food into fractions including moisture, ash, protein, lipids and minerals. Seafood contains high levels of these important components compared to other meats (reviewed in Tacon & Metian, 2013) and is therefore viewed as highly nutritious, and key to human health and well-being (FAO/WHO, 2011; Lloret *et al.*, 2016; Simopoulos, 2002). Oysters, in particular, are a popular and well-known natural source of these nutrients (Asha *et al.*, 2014; Cochet *et al.*, 2015; Orban *et al.*, 2004; Pogoda *et al.*, 2013; Sprague *et al.*, 2017).

In 2015, global oyster production exceeded 5.4 million tonnes, and was valued at >US$4 billion. In the UK, oysters are one of the major aquaculture species (Pinnegar *et al.*, 2017) with ~1600 tonnes produced in 2015, and worth more than US$6.4 million. Yet there is increasing concern over the long-term future of shellfish production due to the effects of environmental stressors associated with rising atmospheric CO_2 levels such as warming and marine heat waves, falling levels of seawater carbonate and oxygen plus rising sea levels and increased storminess (Branch *et al.*, 2013; Cooley *et al.*, 2015; Dupont *et al.*, 2014; Ekstrom *et al.*, 2015; Lemasson *et al.*, 2017b). Ocean acidification and sea-surface warming are changing animal physiology and affecting the quality of seafood (Dupont *et al.*, 2014; Tate *et* *al.*, 2017). In oysters, the effects of ocean acidification are already being detected (Lemasson *et al.*, 2017a), with several hatcheries experiencing declines in production, jeopardising economic revenues and necessitating adaptive actions (Barton *et al.*, 2015; Cooley *et al.*, 2017). However, physiological effects of ocean acidification and warming in oysters appear species-specific (Lemasson *et al.*, 2018).

To date, there has been limited consideration of potential changes in the quality of shellfish under warming and acidification. The few published studies have shown changes, such as reductions in protein and lipid content, and reductions in omega-3 fatty acids (Ab Lah *et al.*, 2018; Clements *et al.*, 2018; Tate *et al.*, 2017; Valles-Regino *et al.*, 2015). A better understanding is needed if we are to shed light on the future of shellfish aquaculture. Here, using two economically and commercially important species of oysters, *Magallana gigas* – a non-native introduced species – and *Ostrea edulis* – a native species –, we tested if ocean acidification and warming conditions predicted under future climate scenarios has the potential to impact seafood nutritional quality. We also consider how condition indices – a widely used metric in aquaculture for evaluating health and value of bivalves because they are correlated with meat yield (Knights, 2012; Orban *et al*. 2002, 2006) – might change under ocean acidification and warming scenarios.

2 Methods

2.1 Organism collection and treatments

Collection of organisms, acclimation, treatments, and mesocosm set-up followed the protocol described in Lemasson *et al.* (2018). Following 14 days of acclimation to laboratory conditions (16°C, salinity 33, ~400 ppm pCO_2, 12:12 dark:light cycle, fed *ad libitum* with a mixed algal diet (Shellfish Diet, 1800; Reed Mariculture)), each oyster was placed in its own 3 L experimental tank and exposed for 12 weeks to three pCO_2 concentrations (ambient ~400 ppm, intermediate ~750 ppm, elevated ~1000 ppm), and two temperatures (control 16.8 °C, elevated 20 °C) in an orthogonal experimental design (*M. gigas* n= 4; *O. edulis* n=8 individuals per treatment). This design aimed to simulate current and future ocean acidification and warming scenarios, using scenarios in line with conditions predicted by the IPCC (IPCC, 2013) and for the UK for mid- to end-century (see also Lemasson *et al.*, 2018). Throughout the study, oysters were fed *ad libitum* with a mixed algal diet (Shellfish Diet, 1800; Reed Mariculture).

Temperature, salinity, and pH were measured daily in all replicate tanks. Salinity was measured using a handheld refractometer (D&D The Aquarium Solution Ltd, Ilford, UK) and temperature measured using a digital thermometer (TL; Fisher Scientific, Loughborough, UK). pH was measured using a microelectrode (InLab® Expert Pro-ISM; Mettler- Toledo Ltd, Beaumont Leys, UK) coupled to a pH meter (S400 SevenExcellence™; Mettler-Toledo Ltd, Beaumont Leys, UK), following calibration with NIST traceable buffers. pH in the header tanks was also monitored (data not shown). Total Alkalinity (AT) was measured once a week in each of the replicate tanks. 125 mL water samples were transferred to borosilicate bottle with Teflon caps and poisoned with 30 µL of saturated $HgCl_2$ solution (0.02% sample volume) before being kept in the dark until measurement by automatic Gran titration (Titralab AT1000 © Hach Company). Partial pressure of carbon dioxide (pCO_2) and saturation states of calcite and aragonite (Ωcalcite and Ωaragonite), were calculated at the end of the experiment using CO_2 SYS (Pierrot *et al*., 2006), employing constants from Mehrbach et al. (1973) refitted to the NBS pH scale by Dickson and Millero (1987) and the KSO_4 dissociation constant from Dickson (1990).

Table 1: Seawater chemistry for *Magallana gigas* and *Ostrea edulis* in each treatment. Data shown are means (±SD) values. T=Temperature in °C. ppm= parts per million. Ω_{Ca}= saturation state of calcite. Ω_{Ar}= saturation state of aragonite. A_T= Total alkalinity in mmol/kg seawater.

	Treatment	**Measured**				**Calculated**		
	(pCO$_2$ + Temperature)	pH	T	A_T	S	pCO$_2$	ΩAr	ΩC$_a$
Magallana gigas	Ambient + Control	7.79±0.10	16.9±0.2	2.13±0.32	33.9±1.1	597.2±146.1	1.70±0.32	2.64±0.50
	750 ppm + Control	7.67±0.12	16.9±0.2	2.13±0.33	33.9±1.2	816.9±296.4	1.4±0.36	2.10±0.55
	1000 ppm + Control	7.55±0.10	16.8±0.2	2.13±.032	33.9±1.2	1174.6±420.9	0.99±0.22	1.53±0.34
	Ambient + Elevated	7.84±0.10	20.4±0.3	2.32±0.29	34.3±1.2	669.7±155.9	2.02±0.31	3.11±0.47
	750 ppm + Elevated	7.70±0.11	20.6±0.4	2.33±0.29	34.2±1.1	945.1±275.7	1.60±0.34	2.46±0.52
	1000 ppm + Elevated	7.56±0.10	20.2±0.3	2.34±0.31	34.3±1.2	1376.8±280.8	1.14±0.17	1.76±0.26
Ostrea edulis	Ambient + Control	8.00±0.08	16.5±0.3	3.04±0.18	34.2±0.8	481.4±90.9	3.70±0.65	5.75±1.01
	750 ppm + Control	7.84±0.08	16.6±0.2	3.04±0.19	34.2±0.8	760.1±178.2	2.68±0.51	4.17±0.80
	1000 ppm + Control	7.72±0.16	16.6±0.2	3.00±0.16	34.3±0.7	1053.6±223.3	2.15±1.20	3.34±1.87
	Ambient + Elevated	8.00±0.08	19.8±0.3	2.86±0.15	34.4±0.9	467.9±78.4	3.80±0.65	5.85±1.01
	750 ppm + Elevated	7.90±0.07	20.2±0.5	2.87±0.15	34.4±09	694.7±135.4	2.94±0.47	4.52±0.72
	1000 ppm + Elevated	7.70±0.09	19.8±0.3	2.6±0.22	34.4±0.9	1165.0±226.8	2.01±0.47	3.10±0.73

2.2 Condition index, proximate composition, and energy content

After 12 weeks exposure, oysters were manually shucked and their wet tissue mass (g) was recorded on an electronic balance (Mettler AE240), before being oven-dried at 105°C for 24 hours until constant mass was achieved.

The Condition Index (CI) of each oyster was calculated following the method recommended by Lucas and Beninger (1985) as follows:

$$CI = (dry\ meat\ weight / dry\ shell\ weight) \times 100$$

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Moisture percentage of the meat was calculated for each individual oyster according to the following formula:

$$Moisture\ (\%) = ((\text{Total weight} - \text{Dry weight})/\text{Total weight}) \times 100$$

For each species, following estimation of Condition Index and moisture content for all individuals, the dried meat samples were then pooled by treatment to provide sufficient tissue material for proximate composition and energy content analyses. Pooled samples were homogenised, then ground into a fine powder using a coffee grinder. Complete or partial pooling of specimens from the same treatment or sampling site for biochemical analysis has been reported in several studies (Fernandez *et al.*, 2015; Marin *et al.*, 2003; Soto-Jiménez *et al.*, 2001). While not allowing individual comparisons, this approach provides nutritional information at the population level. The following assays were performed in triplicate.

Ash content (a measure of the total amount of minerals present within a food) was determined using 500mg of dried tissue samples and an adaptation of the Association of Official Agricultural Chemists official method (AOAC, 1995). Lipid content was determined by continuous extraction of fat from 2 g material using petroleum ether as a solvent following the Soxhlet method (Luque de Castro & García-Ayuso, 1998; Manirakiza *et al.*, 2001) in a Soxtherm Rapid Extraction Unit (C. Gerhardt GmbH & Co. KG). Total protein content was determined using the Kjeldahl method (Kjeldahl, 1883) on ~150 mg samples with a Gerhardt Kjeldatherm digestion block, a Gerhardt Turbosog scrubber unit and a Gerhardt Vapodest 50s distillation unit (Gerhardt Laboratory Instruments, Bonn, Germany). Glycogen content was determined indirectly by calculating carbohydrate content using the above results for moisture, ash, lipid, and protein content following Maclean *et al*. (2003) as follows:

$$Carbohydrates\ (\%) = 100 - (\%\text{M} + \%\text{A} + \%\text{L} + \%\text{P})$$

Where:

C=carbohydrate, M=moisture, A=ash, L=lipid, and P=protein. All values used were as percentage of total weight.

Caloric (energy) content was measured as gross energy content ($kJ.g^{-1}$) by bomb calorimetry using an isoperibol oxygen bomb calorimeter (Parr Instrument Company, Moline, Illinois, USA) on ~1 g of material per sample.

2.3 Macro and micro-minerals

Macro-mineral (calcium [Ca], potassium [K], magnesium [Mg], sodium [Na]) and micro-mineral (copper [Cu], iron [Fe], zinc [Zn]) content was determined using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES; iCAP 7000series Thermo Scientific) following standard protocols as reported elsewhere (Rider *et al.*, 2010). The content of the micro-mineral selenium [Se] was determined using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS; Xseries2, Thermo Scientific) following standard protocols. Mineral composition was determined on ~100-150 mg of material per sample. Before use, both ICP-OES and ICP-MS were calibrated using mixed, matrix-matched standards (0–100 $\mu g.L^{-1}$) prepared from certified Aristar plasma emission grade solutions. Quality control was assured by carrying out accuracy checks using a known standard or blank every 10 samples during the analysis. Also, 2% internal iridium and indium standards (P/N/4400-013 CPI, Quality control standard 26) were added to each sample. DORM-3 (dogfish certified reference material − CRM) from National Research Centre Canada (NRCC) was used to verify the digestion procedure as reported elsewhere (Rossbach *et al.*, 2017).

2.4 Statistical analyses

The logistical constraints of the experimental infrastructure meant oyster species had to be tested sequentially. There were natural variations in the chemistry of the seawater used between experiments due to natural seasonal fluctuations in seawater properties combined with differences in atmospheric partial pressure (barometric pressure). These fluctuations in pCO_2 also led to fluctuations in CO_2 adsorbed by the seawater. The resulting pCO_2, and pH, conditions were therefore different between experiments, but also within experiments were different from the expected levels of ~400ppm, ~750ppm and ~1000ppm. Nevertheless, the effect size (magnitude of difference in pCO_2 and pH between treatments within experiments) were comparable. These constraints prevented a formal comparison of the two species in a factorial design, so these were analysed separately. Analyses were performed using R Version 3.2.5 (R Core Development Team, 2018) using the *base*, *MASS*, *stat* and *vegan* packages. $p < 0.05$ was used as statistical threshold.

Condition Index and Moisture content

Condition index and moisture data (obtained before pooling of the samples) were tested for homogeneity of variances using Levene's test (*car* package). Tests for differences in condition index and moisture content between treatments were done using 2-factor ANOVA with 'temperature' and 'pCO_2' as fixed factors.

Proximate composition, energy content, and mineral composition

As oyster tissues were pooled in order to provide sufficient material to perform these analyses, there were no 'true replicates' (*sensu* Hurlbert, 1984). Triplicate measures of protein, lipid, ash, calorimetry, and mineral analysis were performed to determine within sample variability.Data were pooled, and averages used for statistical analysis to avoid Type I error.

The pooled samples used were therefore homogenised samples of multiple individuals (*M. gigas* n=4; *O. edulis* n=8), and in effect the single values associated with each assay are means without the variances. Analyses were thus performed on these single values.

Calorimetry data were analysed using a 2-way ANOVA with 'temperature' and 'pCO_2' as fixed factors (n=3 per Temperature level; n=2 per pCO_2 level). Due to this experimental design, interactions between the two factors could not be assessed.

To compare proximate and mineral compositions across species, temperature and pCO_2, nonmetric multidimensional scaling (nMDS) coupled with Permutational Multivariate Analysis of Variance (PERMANOVA, Anderson, 2001) was used to evaluate the significance of observed patterns (based on Euclidian distance and 1000 permutations of the data) and test hypotheses related to changes in composition due to experimental treatment.

For tests using ANOVA, when significant differences were present, *post-hoc* Tukey tests were performed to assess differences between treatment levels.

3 Results

3.1 Condition Index and Moisture

Temperature, but not pCO_2, had a significant effect on the condition index of *M. gigas* ($F_{1,12}$ = 12.298; $p<0.01$), with condition index negatively impacted by elevated temperature (Figure 1a). Mean condition index decreased 35% with increased temperature, from ~3.2 (±0.3) at ambient temperature to ~2.1 (±0.20) at elevated temperature. While marginally not significant ($F_{2,18}$=2.97; p=0.07), there were clear differences in condition indexbetween *M. gigas* cultured under different temperature and pCO_2 regimes, with a trend toward increasing condition indexwith increasing pCO_2 at the control temperature, and decreasing condition indexwith increasing pCO_2 at elevated temperature (Figure 1a). The condition indexof *O. edulis* was unaffected by any of the treatments. Moisture – the principal contributor to oyster flesh – was also unaffected by temperature or pCO_2 treatment and ranged between 70-80% for both species (Table 2; Figure 1b).

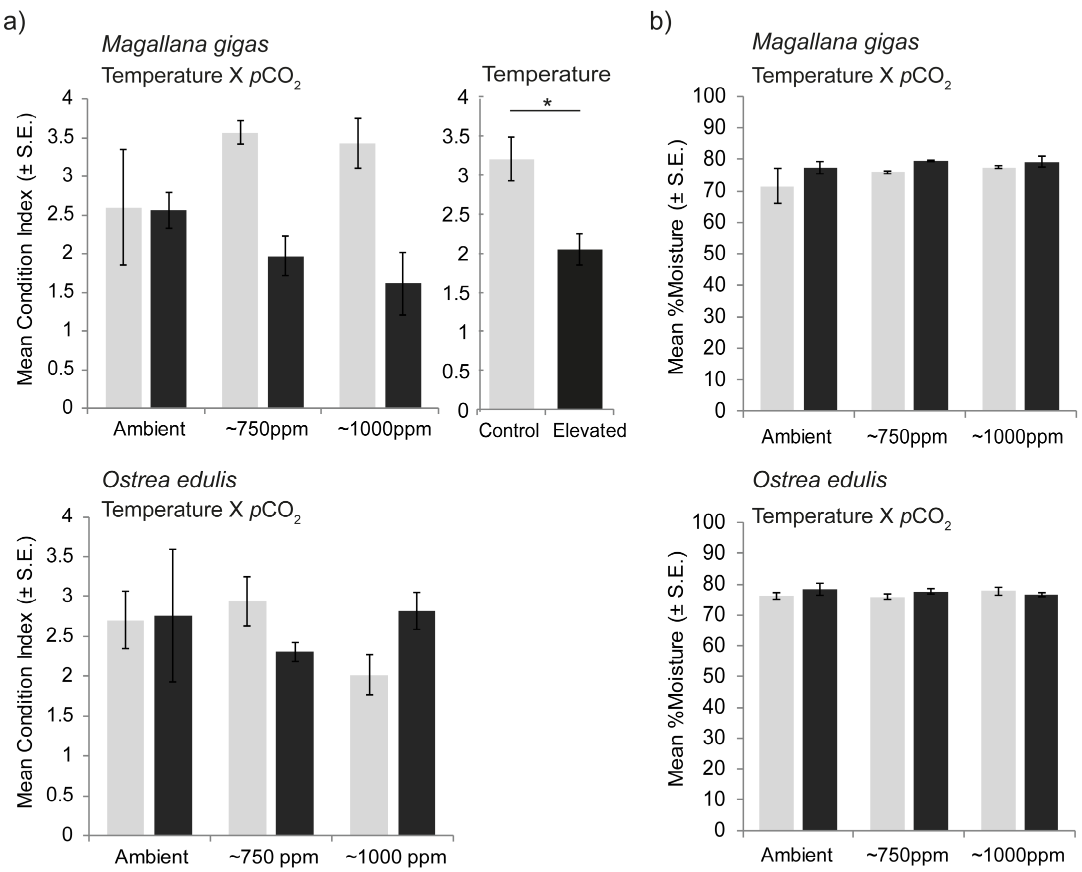

Figure 1 Difference in a) Condition Index and b) moisture content (as % of total weight), of *Magallana gigas* and *Ostrea edulis* across temperature and pCO_2 treatments. ppm= part per million; s.e. = standard error. Grey = control temperature. Black= elevated temperature. * indicates significant difference.

3.2 Proximate composition analysis

There were significant differences in proximate composition with temperature and pCO_2 for both species (*Magallana gigas:* perm $F_{2,24}$=75.41; $p < 0.001$; *Ostrea edulis:* perm $F_{2,24}$=14.37; $p < 0.001$) (Table 2, Figures 2 & 3a-d). For all treatments, after moisture, protein was the second largest component in both *M. gigas* and *O. edulis* under ambient pCO_2 and control temperature, representing 16.6% and 9.9% of the total composition, respectively (Table 2; Figure 2). *Ostrea edulis* was characterised by higher carbohydrates (10.5g per 100g) than *M. gigas* (4.5g per 100g), but *M. gigas* had higher percentages of proteins and lipids (Table 2,). Temperature (Figure 3a-b) and pCO_2 (Figure 3c-d) both led to clear dissimilarity in proximate composition between species, with greater changes apparent in *M. gigas*, largely driven by reductions in proteins and lipids (Table 2, Figure 2) from 16.6g to 11.8g and from 4.8g to 1.4g, respectively. The treatments also appeared to a lesser degree to negatively impact the carbohydrate proportion in *M. gigas*, which dropped from 4.9g to 3.3g (Table 2; Figure 2). There were significant differences in proximate composition between all pCO_2 treatments in *M. gigas*, but in *O. edulis*, there was no difference between oysters cultured in 400 and 1000 ppm, whereas those cultured in intermediate pCO_2 (750ppm) were significantly different (Figure 3c). This was particularly evident in *O. edulis* for the lipid proportions, which were reduced from 1.3g to 0.9g at elevated temperature and intermediate pCO_2 (~750 ppm) (Table 2; Figure 2).

Table 2: Proximate composition of *Magallana gigas* and *Ostrea edulis* under six ocean acidification and warming scenarios. Moisture represents the remaining component, adding up to 100g. In g per 100g sample (wet weight).

	Treatment (pCO$_2$ x T)	Protein	Carbo-hydrate	Lipid	Ash	Moisture
Magallana gigas	Ambient x Control	16.6	4.5	4.8	2.7	71.4
	~750ppm x Control	14.6	4.6	2.9	1.9	76.0
	~1000ppm x Control	12.6	3.9	3.9	2.1	77.5
	Ambient x Elevated	12.8	4.9	2.7	2.2	77.4
	~750ppm x Elevated	13.7	3.3	1.4	2.4	79.6
	~1000ppm x Elevated	11.8	4.3	2.6	2.1	79.2
Ostrea edulis	Ambient x Control	9.9	10.5	1.3	2.1	76.2
	~750ppm x Control	10.3	10.3	1.3	2.1	75.9
	~1000ppm x Control	9.7	8.7	1.5	2.4	77.7
	Ambient x Elevated	8.8	9.3	1.6	2.0	78.3
	~750ppm x Elevated	10.4	8.7	0.9	2.5	77.6
	~1000ppm x Elevated	9.2	11.1	1.3	1.9	76.4

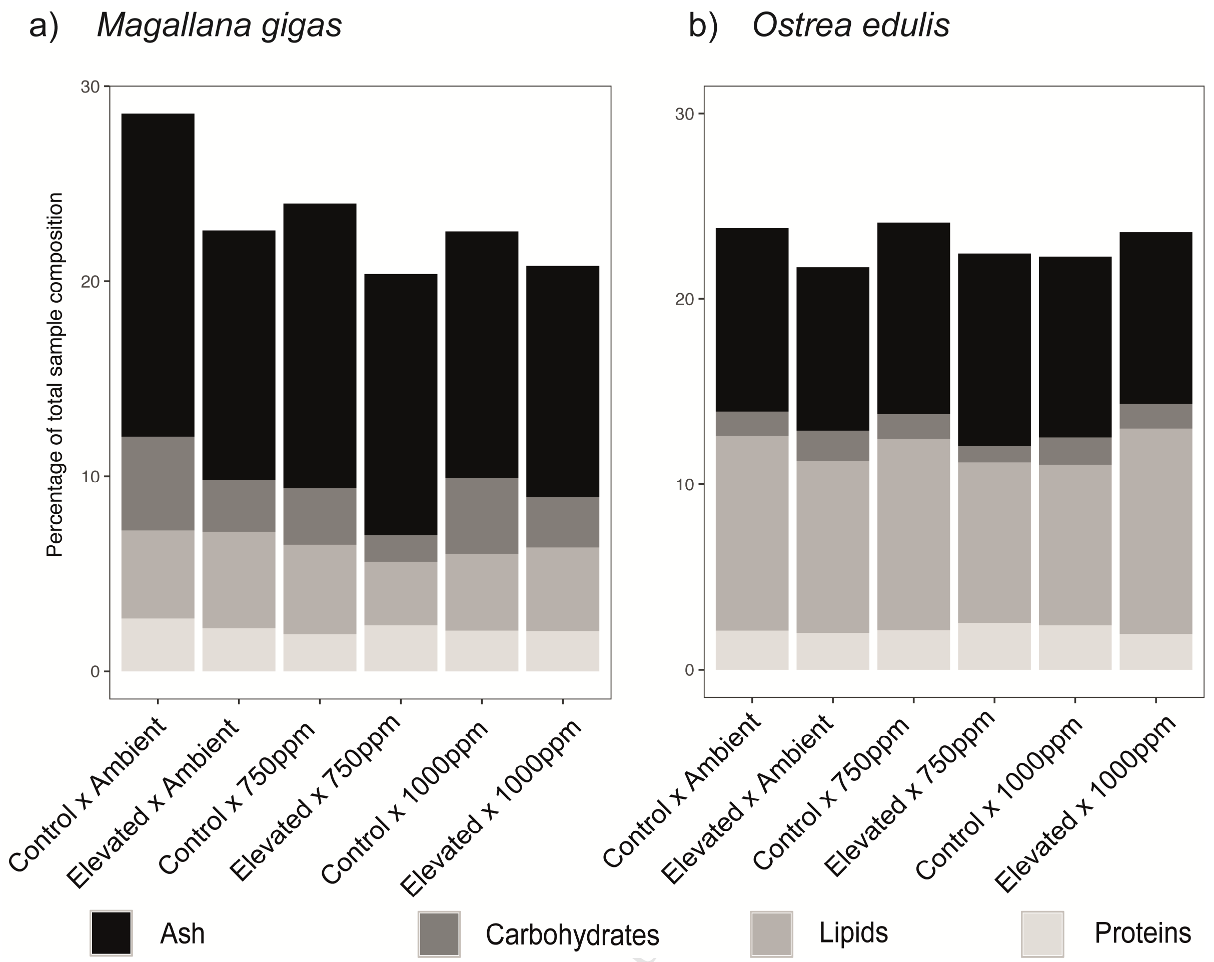

Figure 2: Relative composition of proximate components present in a) *Magallana gigas* and b) *Ostrea edulis* across temperature and pCO_2 treatments. The values for each treatment represent the means of the three procedural replicates of the pooled samples. ppm= part per million.

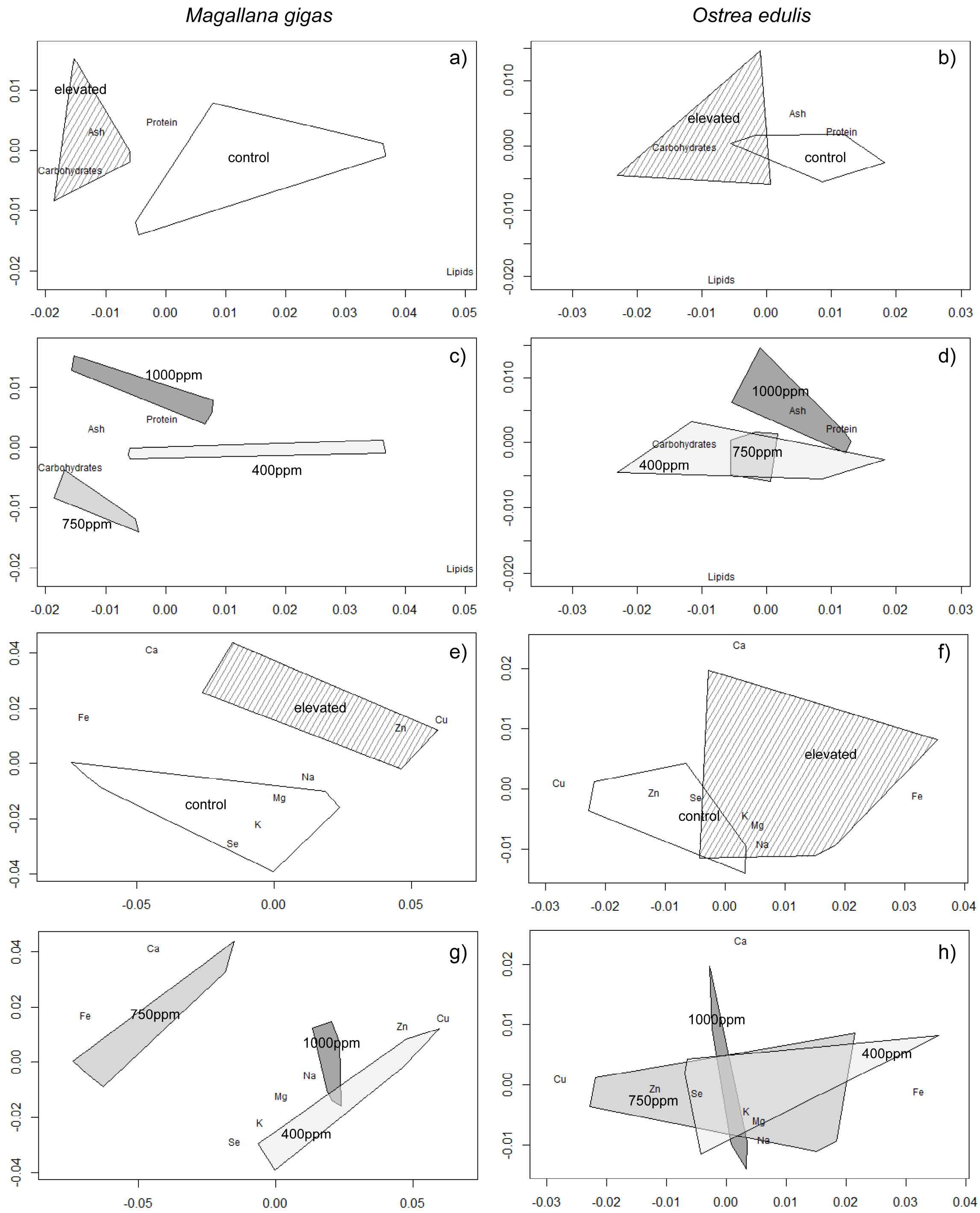

Figure 3: nMDS plots of proximate (a-d) and mineral compositions (e-h) of *Magallana gigas* (left column) and *Ostrea edulis* (right column). Dispersion of points within temperature (plots a, b, e, f) and pCO_2 (plots c, d, g, h) treatments are illustrated using cluster hulls plotted using the R package '*ggplot2*'. Centroids of each proximate composition and mineral components are shown. 2D stress for all plots < 0.08.

3.3 Energy content

There was a 13% reduction in the caloric content of *M. gigas* with temperature and pCO_2 from 20.97 kJ/gDW at control temperature and ambient pCO_2 to 18.41 kJ/gDW at elevated temperature and intermediate pCO_2 (~750 ppm) (Figure 4). The energetic value of *O. edulis* did not change (17.63 kJ/gDW) with treatment (Figure 4).

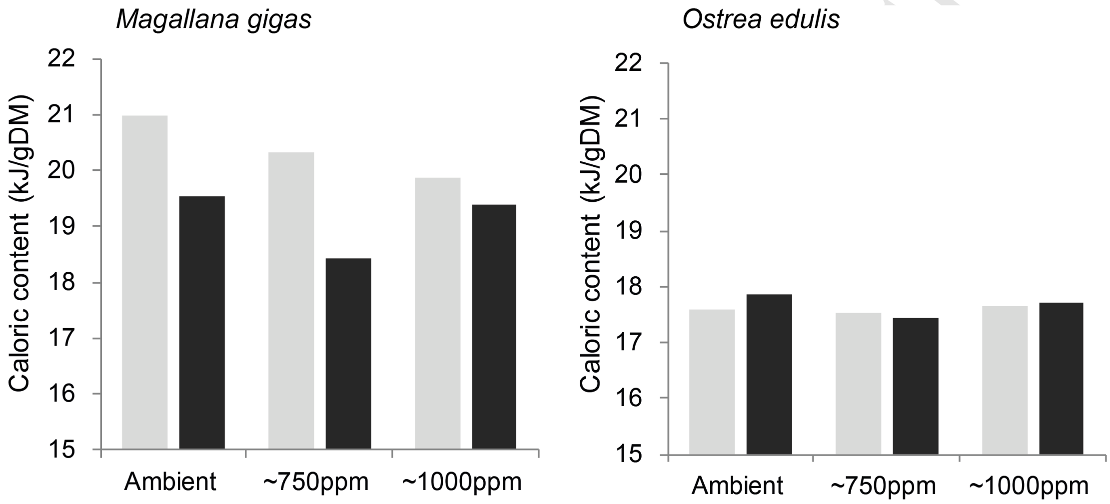

Figure 4: Variations in the caloric content of oysters across temperature and pCO_2 treatments for *Magallana gigas* (left) and *Ostrea edulis* (right). ppm= part per million; Light grey = control temperature. Dark grey= elevated temperature. The value for each treatment represents the mean of the three procedural replicates of the pooled samples, therefore no error bars were obtained. Values are per kg of oyster dry matter (DM).

3.4 Trace elements

There were significant differences in trace elements composition with, temperature and pCO_2 levels for both species (*Magallana gigas:* perm $F_{2,24}$=166.75; $p < 0.001$; *Ostrea edulis:* perm $F_{2,24}$=16.27; $p < 0.001$) (Table 3, Figure 3e-h). *Magallana gigas* was characterised by higher Se, Fe, K, Mg, and Na content than *O. edulis* (Table 3, Figure 3e-h), but *O. edulis* displayed higher Zn levels. Temperature led to clear dissimilarity in the mineral composition of each oyster species, with greater change apparent in *M. gigas* (Figure 3e-fa). pCO_2 also had notable effects, but only on *M. gigas* (Table 3, Figure 3g-h). Overall, the mineral composition of *M. gigas* was clearly affected by the treatments (Table 3), with notable increases in Cu and Zn content, and decreases in Fe and Se contents.

Table 3: Mineral composition of *Magallana gigas* and *Ostrea edulis* under six ocean acidification and warming scenarios. T= temperature. ppm= part per million. Ca=calcium; Cu=copper; Fe=Iron; K=potassium; Mg=magnesium; Na=sodium; Zn=zinc; Se=selenium. All values are in mg.kg^{-1}, except Se which is in μg.kg^{-1}.

	Treatment (pCO_2 x T)	**Ca**	**Cu**	**Fe**	**K**	**Mg**	**Na**	**Zn**	**Se**
Magallana gigas	Ambient x Control	1250.0	64.7	205.5	322.1	847.1	4626.3	450.3	537.1
	~750ppm x Control	492.8	60.0	35.7	268.1	553.5	3101.7	404.9	313.1
	~1000ppm x Control	618.1	149.0	54.0	270.2	676.7	3811.5	749.2	459.7
	Ambient x Elevated	1409.6	92.5	74.7	219.2	509.6	3111.3	566.8	248.8
	~750ppm x Elevated	544.5	202.0	57.7	260.4	793.6	5476.6	1063.2	309.4
	~1000ppm x Elevated	719.1	143.5	69.4	214.2	609.5	3969.1	798.3	314.0
Ostrea edulis	Ambient x Control	1567.2	196.2	48.4	277.4	546.0	3747.0	1077.3	263.6
	~750ppm x Control	1535.5	127.1	39.3	253.3	496.8	3390.0	862.9	232.8
	~1000ppm x Control	1353.5	103.0	42.0	270.7	636.7	4571.7	940.0	252.1
	Ambient x Elevated	1267.3	87.6	55.9	257.4	501.3	3503.5	623.8	192.0
	~750ppm x Elevated	1560.6	111.1	64.8	258.8	539.5	3842.1	879.7	256.1
	~1000ppm x Elevated	2142.2	113.4	37.8	258.0	545.5	3672.5	898.1	248.8

4 Discussion

The ability of human society to feed the ever-growing population is a major ongoing concern, particularly as climate change is already negatively impacting food production from both terrestrial and marine environments (Brander *et al.*, 2017; Campbell *et al.*, 2017; UNEP, 2010). Mollusc aquaculture is increasingly recognised as a solution to this issue. Here, following exposure to temperature and pCO_2 levels predicted for 2050 to 2100, we show species-specific variations in the nutritional quality of two commercially important oyster species. Both *O. edulis* and *M. gigas* displayed changes in biochemical (proximate and mineral) composition; in particular *M. gigas* had lower lipid, carbohydrate, and protein levels, but higher copper concentration, which could pose concerns for both future food safety and security.

4.1 Condition Index and Moisture content

Condition indices are widely used in aquaculture to evaluate the overall health and value of bivalves, and select specimens of the highest quality (Knights, 2012; Orban *et al.*, 2006; Orban *et al.*, 2002). These indices are correlated with the meat yield, which declines in bivalves under stressful environmental conditions the require significant energetic expenditure (Orban *et al.*, 2002). The condition indexof *M. gigas* was negatively impacted by elevated temperature but not elevated pCO_2, whereas the condition indexof *O. edulis* was unaffected by any of the treatment conditions, suggesting that the two species did not experience or respond to environmental change in the same way. Changes in condition index reflect the respective changes in feeding and respiration rates to ocean acidification and warming of the two species, as observed by Lemasson *et al.* (2018). In the Lemasson *et al.* (2018) study, *M. gigas* increased its metabolic rate at elevated temperatures and reduced its feeding rate at elevated levels of pCO_2 (~750 ppm), leading to reduced condition index, whereas the metabolic rate and feeding rate of *O. edulis* was unaffected by ocean acidification and warming. These results are in contrast to those of Lannig *et al*., (2010) on *M. gigas* who recorded a decrease of ~20% in condition index between individuals exposed to ambient and elevated pCO_2 (see further discussion on the effects of ocean acidification and warming on bivalves in Lemasson *et al.,* 2018). In bivalves, declines in condition index usually suggest depletion of reserves following energetic reallocation, which can lead to changes in individuals biochemical composition (proximate and mineral) and consequently in their nutritional value (see EFSA NDA Panel, 2014; Tate *et al.*, 2017).

Water constitutes the major part of oysters (Asha *et al.*, 2014). This component is linked to juiciness, which is an important sensory trait of oysters and influences their marketability (Cruz-Romero *et al.*, 2004). Sensory traits, such as juiciness, texture, appearance, odour or taste, are linked to biochemical composition, and have recently been shown to be unchanged in oysters under ocean acidification and warming (Lemasson *et al*., 2017b). As was also observed in *Turbo militaris* (Ab Lah, 2018), here the moisture content of either *M. gigas* or *O. edulis* did not change when exposed to ocean acidification and warming conditions.

4.2 Energetic reserves

Protein, lipids, and carbohydrates constitute the main energy storage compounds in bivalves, which all have important functions in physiological processes, for instance gametogenesis and reproduction (Dridi *et al.*, 2007). By influencing oysters physiology and metabolic responses, environmental conditions, such as ocean acidification and warming, can dictate the accumulation and depletion of energetic reserves in bivalves (Clements *et al.*, 2018)..

Carbohydrates

Carbohydrate, in the form of glycogen, is thought to be the energy reserve present in the highest quantity in bivalves and is used to sustain routine metabolic processes (Anacleto *et al.*, 2014, and references therein). A decline in glycogen content under environmental stress (e.g. hypercapnia, hyposalinity, increased temperature) is common in oysters and can indicate physiological stress (Dickinson *et al.*, 2012). Here, the carbohydrate content of *M. gigas* was reduced under ocean acidification and warming, particularly at ~750 ppm pCO_2 and elevated temperature, but was unaffected in *O. edulis*. Carbohydrate content remained high in *O. edulis* (>8.5% wet weight) compared to *M. gigas* (<5% wet weight). Native bivalve species have previously been shown to have higher glycogen content than non-native and invasive species, this may be a metabolic adaptive strategy to cope with environmental change (Anacleto *et al.*, 2014), and may account for the lack of response of *O. edulis* to ocean acidification and warming here. Given the importance of carbohydrates for oyster maintenance, condition, and their ability to sustain physiological processes, depletion of carbohydrate reserves in *M. gigas* might jeopardize organisms survival in the long term, which aligns with results showing reduced condition (Lemasson et al., 2018).

Proteins

Proteins supply structural elements and have a crucial role in metabolic reactions. Under sustained stress, bivalves can catabolise proteins to mobilise energy once carbohydrates and lipids have been depleted (Barber & Blake, 1985). *Magallana gigas* and *O. edulis* were both high in protein, but under ocean acidification and warming *M. gigas* displayed important reductions. A similar response was shown in the whelk *Dicathais orbita* , large declines (>50%) in protein content under ocean acidification and warming (Tate *et al.*, 2017), but in *Mytilus edulis* (Clements *et al*., 2018) and *T. militaris* (Ab Lah *et al.*, 2018*)*, no reductions in protein were observed suggesting taxon-specific responses.

Lipids

The array of lipids in molluscs, with a low proportion of saturated fatty acids and high proportion of polyunsaturated fatty acids (including Ω-3), offer numerous health benefits to people (Sprague *et al.*, 2017). Here, *M. gigas* contained higher levels of lipids (~1.4-4.8% wet weight) compared to *O. edulis*, in the range reported elsewhere (Cochet *et al.*, 2015; Pogoda *et al.*, 2013; Shpigel *et al.*, 1992). In contrast, *O. edulis* used in this study were relatively poor in crude lipids (~0.8-1.6% wet weight), with values below those reported elsewhere (Pogoda *et al.*, 2013). Ocean acidification and warming scenarios led to a decrease in lipid content in both species, particularly notable at ~750 ppm pCO_2. Larger absolute reductions occurred in *M. gigas* (30%; wet weight percentage) which possessed a higher baseline lipid content under control conditions, but larger percentage reductions were apparent in *O. edulis* (~50%; wet weight percentage). Reductions in total lipid content and differences in fatty acid composition (including decreases in polyunsaturated fatty acids) under ocean acidification and warming have been shown in other molluscs (Ab Lah *et al.*, 2018; Tate *et al.*, 2017; Valles-Regino *et al.*, 2015 but see Clements *et al*. 2018), with variation attributed to differential deposition and energy use rates between species (Child & Laing, 1998; Pogoda *et al.*, 2013).

Although carbohydrates (and especially glycogen) are often the preferred source of energy for oysters, species-specific differences may exist (see discussion in Pogoda *et al.*, 2013). It has previously been suggested that *O. edulis* preferentially use lipids whereas *M. gigas* use proteins as their principal energy source for metabolic activity when subjected to food limitation (Child & Laing, 1998). Here, both oyster species when exposed to ocean acidification and warming appeared to use lipids and carbohydrates as their primary source of energetic reserves, but to a lesser extent by *O. edulis*, possibly because they did not have important lipid reserves in the first place. Depletions of energetic reserves were indeed particularly apparent for *M. gigas*, with additional reductions in proteins reflected in the reduced condition index and caloric content, especially at intermediate pCO_2 level. The differential use of energetic reserves by oysters is therefore likely a consequence of the differential physiological stress endured when exposed to ocean acidification and warming conditions (Lemasson et al., under review).

4.3 Mineral content

Seafood quality also varies based on the proportion (total ash) and composition of inorganic minerals. In particular, minerals are an essential component of a healthy diet in humans (EFSA NDA Panel, 2014). Here, the two oyster species displayed similar ash content (~1.9-2.6%), which was unimpacted by ocean acidification and warming. In fact, a modest increase in ash content in *O. edulis* under elevated pCO_2 might indicate mineral accumulation within the tissue. Ab Lah *et al.*, (2018) have also found no changes in ash content of *T. militaris* under ocean warming and acidification.

Although nutritionists often focus on macronutrients, such as calcium (Ca) and magnesium (Mg), which are beneficial for teeth and bones (Lambert *et al.*, 2017), there is an increasing understanding of the dietary benefits of trace minerals (FAO, 2016). For instance, potassium (K)-rich foods are considered particularly healthy; selenium (Se) strengthens the immune system and reduces oxidative stress in tissue (Rayman, 2000); and zinc (Zn) and iron (Fe) are critical for stamina and disease resistance (Knez *et al.*, 2017; Solomons & Schümann, 2017). Moreover, micronutrient deficiencies afflict an enormous proportion of the population. For instance, over 2 billion people are diagnosed as iron-deficient, and an estimated 800,000 children die every year from zinc deficiency (FAO, 2016).

In this study, large differences in the levels of macro and micro-nutrients between *M. gigas* and *O. edulis* were evident, which is unsurprising as species-specific differences in mineral composition is common in bivalves (Bray *et al.*, 2015). Notably, *M. gigas* exposed to the current climate conditions were relatively high in K, sodium (Na), Mg, Fe, and Se when compared to *O. edulis*, which was high in Zn. While the values presented here for macro- and micro-minerals are within the ranges described in other studies on oysters and may not be locally dependent (Se: Cantillo, 1998; Fe: Diaz Rizo *et al.*, 2010; Na, K, Mg, Fe, Se: Orban *et al.*, 2004), concentrations of copper (Cu) and Zn in this study were significantly higher than those commonly found in literature (Cantillo, 1998). High Cu and Zn contents have been described for oysters growing in contaminated locations associated with mining and harbour activities (Diaz Rizo *et al.*, 2010; Frias-Espericueta *et al.*, 2009). Plymouth Sound – the location of oyster collection for this study – has a long history of mining that has led to significant contamination of its waters and substrates (see Knights *et al.*, 2016 and references therein), which could explain the elevated Cu and Zn levels obtained here.

Here, exposure to ocean acidification and warming conditions led to species-specific changes in the concentration of those minerals, with the mineral composition of *M. gigas* being especially affected. A recent study found increased levels of Zn in *T. militaris* exposed to ocean acidification and warming conditions, but without changes in other micro- and macro-elements concentrations (Ab Lah *et al.*, 2018). Here, the reductions in Ca, Fe, and Se content in *M. gigas* exposed to ocean acidification and warming to levels similar or lower than *O. edulis*, coupled to the accumulation of Cu, represent a measurable change to its nutritional value, which could have nutritional and safety implications. Copper, along with other trace metals such as arsenic, copper and lead, can become toxic to marine organisms in high concentrations (Götze *et al*., 2014; Moreira *et al.*, 2016), and threaten human health through seafood ingestion (Bhupander *et al*., 2011; Han *et al.*, 1998). Since bivalves are filter feeders, they readily accumulate metals present in the surrounding waters into their edible tissue (Lu *et al.*, 2017; Raposo *et al.*, 2009). This process can be modulated by ocean acidification, for instance enhancing the bioaccumulation of Cu in oysters (Belivermiş *et al*., 2015; Götze *et al.*, 2014; Hawkins & Sokolova, 2017; Ivanina *et al.*, 2015; Ivanina *et al.*, 2016). While Cu accumulation under ocean acidification and warming can come at metabolic costs to organisms (Hawkins & Sokolova, 2017), the implications for human consumption are still unclear. For instance, in Plymouth Sound where background levels are already high, further bioaccumulation of potentially harmful minerals, such as Cu or Zn, in *M. gigas* could exceed safe levels for consumption.

4.4 Implications for food security and aquaculture management

Our results suggest that the nutritional quality of *M. gigas*, but not *O. edulis*, is likely to be affected by short-termwarming and acidification of coastal seawater caused by CO_2 emissions. These changes include reduced proteins, lipids, energetic value, as well as changes to their essential mineral contents. Oysters are seldom a major contributor to human diet, however islands and countries with little agricultural land rely on wild-caught seafood and aquaculture for protein (Cooley *et al*., 2012). Should the changes observed in oysters be widespread in seafood species, then the nutritional benefits of seafood to human health and its role in food security may be further compromised (Cooley *et al.*, 2012; Ding *et al.*, 2017). Given the need for additional and sustainable sources of proteins, the current exponential expansion of the aquaculture industry is inevitable; nevertheless a careful evaluation of this industry as well as the development of appropriate mitigations plans (Clements and Chopin, 2017) are needed to ensure that aquaculture is a wise investment in the face of ocean acidification and warming. Diversifying the target species and promoting those currently under-utilized may supplement the industry with 'novel' sources of protein. However, this in practicality might face new challenges, such as selecting species that also thrive under aquaculture conditions and avoiding selecting non-native species (Arismendi *et al.*, 2009), and might require strategic management plants. In order to optimise protein supply and secure socio-economic benefits of mollusc aquaculture, research needs to focus on identifying and selecting native aquaculture species that are resilient to future climate conditions, and able to retain their beneficial nutritional properties (Cooley *et al.*, 2012; Sato *et al.*, 2018), without introducing new challenges.

Our results suggest *M. gigas* is at higher risk of reduction in nutritional quality than its native counterpart *O. edulis* under future ocean acidification and warming scenarios. In the UK, a reduction in the nutritional quality of oysters may not quickly be recognised by consumers, but lower energetic reserves and condition of *M. gigas* may hold an economic relevance to the aquaculture industry, since this species currently represents >90% of the production (Humphreys *et al.*, 2014). Additionally, the biochemical composition can dictate meat appearance, aroma, taste and texture (Cochet *et al.*, 2015; Fratini *et al.*, 2013), and any changes in biochemical composition occurring because of ocean acidification and warming can impact on the sensory quality (Lemasson *et al.*, 2017b). Therefore changes in biochemical composition under ocean acidification and warming can influence the consumer appeal for the product, reducing the demand for it and depressing its economic value (Cooley *et al.*, 2012). As such, the UK aquaculture industry might need to reconsider the management strategy for the future (Fernandes *et al.*, 2017; Jennings *et al.*, 2016) and consider a shift in focus toward species more robust to climate change, such as *O. edulis*, in order to secure future food provision and economic revenue.

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Highlights: (3-5)

- Ocean acidification and warming can reduce oysters nutritional quality
- Changes to nutritional composition were more pronounced in the introduced species
- Oysters displayed decreased protein, lipid, and carbohydrate contents
- Multifaceted implications for the aquaculture sector and future food security